Acknowledgements.—The author wishes to express his gratitude to two *Streptanthus* specialists, Mr. Freed W. Hoffman and Dr. John L. Morrison, for their continued advice and criticisms. Dr. C. Leo Hitchcock kindly read the manuscript. Thanks are due also to the curatorial staffs of the University of California and the United States National Herbarium for loans of specimens.

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STUDIES OF THE POLLEN GRAIN AND POLLEN TUBE IN CERTAIN MALVACEAE

R. M. Datta

The pollen grains of all Malvaceae in which they have been studied are round with spinescent outgrowths of varying shapes and lengths distributed uniformly on the exine wall (Wodehouse, 1935; Zander, 1935; Lang, 1937; Erdtman, 1952). A varying number of roundish conspicuous apertures is distributed evenly upon the exine surface. Concerning these apertures, Wodehouse (1935) remarks, "Though their shape and their function of serving as places of exit for the pollen tube prompt us to call them germ pores, there is much evidence to show that such apertures are morphologically furrows, which have become so shortened that they coincide in extent with their enclosed germ pores."

Amici (1830), the discoverer of the pollen tube, recorded polysiphonous germination of the pollen grain in *Hibiscus Trionum* and *H. syriacus;* in the latter species, some grains gave rise to twenty to thirty tubes. Guignard (1904) corroborated him after *in vivo* studies of *H. Trionum,* and found that only one tube plays a part in fertilization. Stenar (1925) found

in *Althaea rosea* ten tubes per grain, and in *Malva neglecta,* fourteen. Lang (1937) found five to ten tubes per grain produced by *Anoda cristata* and *Lavatera cachmeriana, in vitro.* Iyenger (1938) reported two tubes per grain in diploid Asiatic cotton (*Gossypium herbaceum*) and in tetraploid American cotton (*G. hirsutum*). He concluded that the frequency of two tubes is greater in the tetraploid American types than in the diploid Asiatic ones, and noted branching of the tubes, in the styles only. He noted polysiphonous germination also in *Hibiscus vitifolius.* Purewall and Randhawa (1947) found pollen grains of *H. esculentus* to germinate thirty minutes after they were placed in culture media. They grew more rapidly in culture media than under moist conditions. As many as six tubes were produced from some grains; branching of the tubes occurred both in culture media and on stigmatic surfaces.

The purpose of the studies here reported was to confirm and extend the foregoing observations.

Acknowledgments

Grateful thanks are offered to Mr. K. N. Agha, Secretary, Indian Central Jute Committee, Calcutta, for kindly pemitting this work to be carried on in the Jute Agricultural Research Institute, Nilganj, near Barrackpore, 24 Parganas, and to Dr. G. I. Patel, Cytogeneticist of the Institute, for his interest and for making facilities available. I am also obliged to Dr. P. K. Sen, Khaira Professor of Agriculture and Head of the Department of Agriculture, Calcutta University, for giving permission and facilities to complete the studies.

Materials and Methods

The seventeen species, varieties, or races, upon which studies were made, are listed in Table 1.

A medium for *in vitro* studies was prepared as follows: 0.5 g. powdered agar agar and 1.0 g. sucrose were dissolved in 25 cc. boiling water. After cooling to 35°C., 0.5 g. powdered gelatin was added and dissolved by stirring. The mixture was filtered through muslin into test tubes plugged with cotton and was sterilized by suspending the tubes in boiling water for several minutes.

Drops of this medium were smeared on clean slides by the technique used in making blood films. The slides were dusted with pollen grains from flowers immediately after anthesis and placed in moist chambers consisting of large Petri dishes.

For *in vivo* studies, flowers were emasculated and bagged. On the following morning they were pollinated from fresh flowers of the same species. After 30–45 minutes, the styles were fixed in acetic-alcohol (1:1). On the following day they were transferred for storage to 70 per cent alcohol. When they were to be examined, they were boiled briefly in lactophenol, cooled, stained for 5 minutes in 1 per cent acid fuchsin, and squashed on the slides.

Studies of the sterility of pollen of *Hibiscus cannabinus* (the American race), *H. radiatus,* and *H. Sabdariffa,* were made late in November of 1954, when the two former species were near the end of their flowering season. Temporary mounts of the pollen of each species were made in aceto-carmine. Stained and unstained grains were counted. The diameters of fifty grains of each species were determined.

Observations

When one or more tubes grow forth from a grain, they contain at first hyaline cytoplasm which shows rapid circulatory movement. Afterwards, starch granules start flowing out of the pollen grain, gradually and ultimately packing up the pollen tubes, with their branches and ramifications, if present and making them turgid. When stained with iodine-potassium-iodide solution, the tubes become deep blue in color.

The tube nucleus and the generative cell or sperms are found in only one tube from a particular grain. As in the generality of pollen tubes, the tube nucleus lies nearer the tip than the generative cell. The generative cell follows the contour of the tube, but the tube nucleus sometimes bulges out slightly. Germination within the anther loculi, which has been observed in the tiliaceous genus *Corchorus* by Datta, 1956, has not been observed in Malvaceae.

Our observations on the occurrence of multiple and branching pollen tubes are given in Table 1.

The pollen grains of *Abutilon Theophrasti, Urena lobata,* and *Althaea rosea* failed to germinate under the conditions provided; those of *Abutilon Avicennae* germinated *in vitro,* but not within one half hour on the stigmas. In *Hibiscus esculentus,* germination was notably rapid, occurring *in vitro* within about five minutes at any period of the day; this shows that the grains remain viable throughout the day; and growth of the tubes was so rapid as to be easily perceived with the low power of the microscope.

Germination through more than one aperture was observed in every species in which germination took place, with the exception of *Malachra capitata.*

Branched pollen tubes were observed in *Hibiscus vitifolius, H. esculentus, H. Sabdariffa, H. populneus, H. cannabinus* (the Indian, Ibadan, and Nigerian races, but not the American) and in F_1 hybrids of *Hibiscus radiatus* and *H. cannabinus.* They were not observed in any of the other species. Since *Hibiscus cannabinus* (Indian) shows branching of the pollen tubes while *H. radiatus* does not, the appearance of this character in the hybrid suggests that it may be dominant.

Pollen of *Hibiscus radiatus* and *H. cannabinus* collected near the end of their period of flowering showed a low percentage of fertility, while *H. Sabdariffa,* still in full flower, was producing pollen of high fertility (Table 2). Ferguson (1924) and Kostoff (1932) have found the age of flowers to affect the fertility of pollen.

TABLE 1. Observations of germination of pollen grains, and of multiple and branching pollen tubes in certain Malvaceae, with references to the illustrations.

Species	Largest number of tubes observed from one grain		Branching	Illustrations
	in vitro	*in vivo*		
Hibiscus vitifolius	11	6	rare	Figs. 8, 9
H. esculentus	26	12	profuse	Figs. 1, 2, 3
H. Sabdariffa	9	8	frequent	Fig. 4
var. *altissima*	3		none	
H. radiatus	6		none	
H. populneus (*Thespesia populnea*)	5		rare	
H. cannabinus (Indian)	11	17	slight	Figs. 5, 6, 7
H. cannabinus (Ibadan)	9		rare	
H. cannabinus (Nigeria)	5		rare	
H. cannabinus (American)	8		none	
H. radiatus × *H. cannabinus* (F_1 plants)	4		rare	Fig. 12
Abutilon Avicennae	3	no germination	none	
A. Theophrasti	no germination	no germination	none	
Sida rhombifolia	2	2	none	Fig. 10
Malachra capitata	1	2	none	Fig. 11
Urena lobata	no germination	no germination		
Althaea rosea	no germination	no germination		

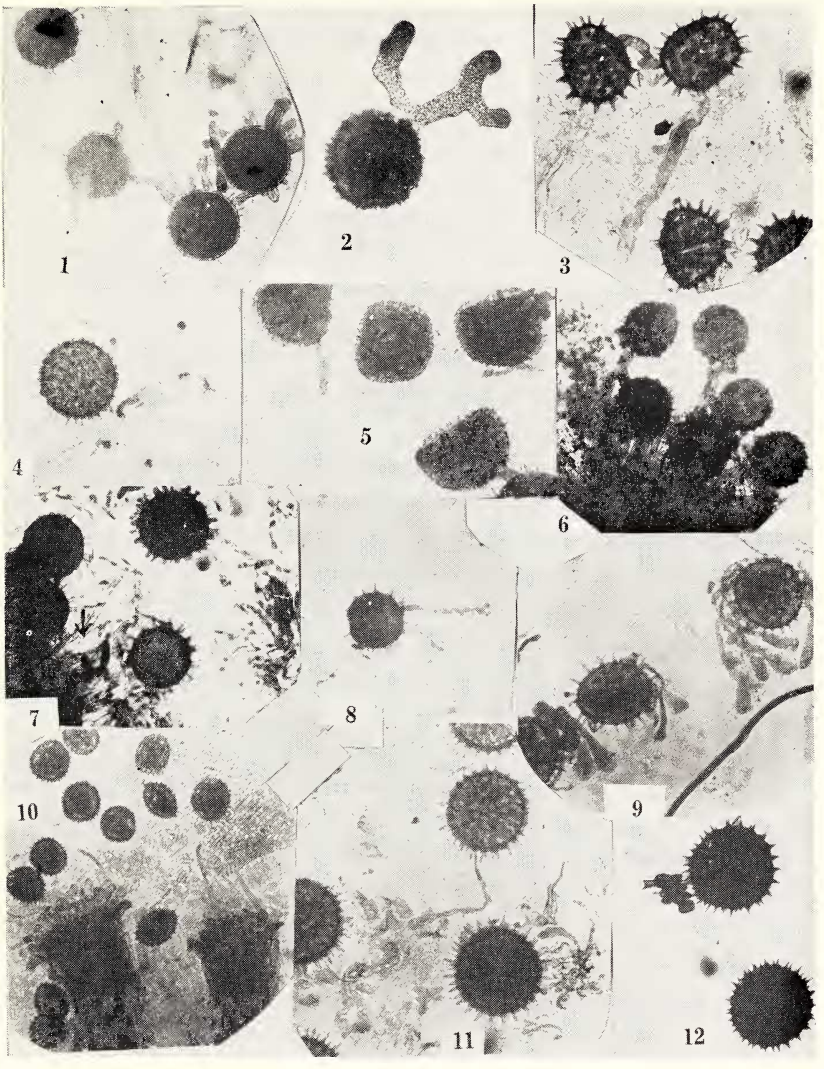
The average diameter of pollen grains of *H. Sabdariffa* as we have determined it, namely 127.9μ, is distinctly smaller than as reported by Lang (1937), namely 145.5μ.

TABLE 2. Observations of fertility and dimensions of pollen grains of three species of *Hibiscus*.

Species	Number of pollen grains examined	Number of stained grains	Number of non-stained grains	Percentage sterility	Range of diameters	Average diameter of 50 grains
H. radiatus	383	179	204	53.26	98.4 –139.4μ	120.96μ
H. cannabinus	450	163	287	63.76	131.12–188.6μ	153.5 μ
H. Sabdariffa	396	353	43	10.86	98.4 –147.6μ	127.9 μ

SUMMARY

Production of pollen tubes from more than one aperture of the pollen grain has been observed in *Hibiscus vitifolius, H. esculentus, H. Sabdariffa* and its variety *altissima, H. radiatus, H. populneus, H. cannabinus,* first generation hybrids of *H. cannabinus* and *H. radiatus, Abutilon Avicennae,* and *Sida rhombifolia.*



Figs. 1–12. Germination of pollen of Malvaceae: 1, 2, *Hibiscus esculentus in vitro;* 3, *H. esculentus in vivo*; 4, *H. Sabdariffa in vivo*; 5, *H. cannabinus* (Indian) *in vitro*; 6, 7, *H. cannabinus* (Indian) *in vivo*; 8, *H. vitifolius in vitro*; 9, *H. vitifolius in vivo*; 10, *Sida rhombifolia in vivo*; 11, *Malachra capitata in vivo*; 12, F_1 hybrid of *H. radiatus* × *H. cannabinus in vitro.*

Branched pollen tubes have been observed in *Hibiscus vitifolius, H. esculentus, H. Sabdariffa, H. populneus, H. cannabinus* (Indian, Ibadan, and Nigerian races), and first generation hybrids of *H. cannabinus* and *H. radiatus*.

Low fertility of pollen observed in *H. cannabinus* and *H. radiatus* is believed to have been caused by lateness of season.

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A NEW SPECIES AND SOME NOMENCLATURAL CHANGES IN SOLANUM, SECTION TUBERARIUM

Donovan S. Correll

In 1852, Dunal described *Solanum lycopersicoides,* no named because of its close resemblance to some of the species in the genus *Lycopersicon,* the common garden tomato. Sometime between 1909 and 1914, Weberbauer collected a plant in an undesignated locality in Peru which, though quite different, superficially resembled *Solanum lycopersicoides*. Again, in 1925 Pennell obtained the same plant at Quive in the Department of Lima.[1] Until now, no apparent attempt was ever made to identify these collections. This distinctive plant is here named in honor of the latter

[1] Since preparing this manuscript, Earl E. Smith, Ramón Ferreyra and I found a solitary sterile plant above Canta in the Department of Lima, Peru, on March 7, 1958.